THE MODIFICATION OF SOME BIOCHEMICAL PROPERTIES OF MUSCLE BY CROSS-INNERVATION*

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Abstract.—Crossing of the nerves leading to muscles with different contraction characteristics leads to biochemical changes related to the modification of the functional properties. Such changes affect the calcium-transporting activity of isolated sarcotubular vesicles, and the amount and isozyme composition of lactic dehydrogenase.

Even within the category of all-or-none responding monosynaptically innervated twitch muscles, there are great differences with respect to contraction velocity, twitch-to-tetanus tension ratio, and active state duration. Buller, Eccles and Eccles[1, 2] and Buller and Lewis[3] have shown these to be ontogenetically determined by the impinging innervation: In the cat, it was found that surgically effected cross-innervation causes a well-nigh complete change of fast-twitch (F-) muscle into a slow-twitch (S-) type, whereas in the reverse case S-muscles would change into F-muscles in terms of twitch duration and twitch-to-tetanus tension ratio, while undergoing only a smaller change in terms of contraction velocity. Buller, Mommaerts, and Seraydarian[4, 5] have discovered that in such cross-innervations the myosin- or myofibrillar ATPase (which is about 4 times faster in the F- than in the S-muscles) changes in accordance with the physiological change, of which indeed it appears to be the cause and explanation.

In this study, we have similarly investigated two other properties of the muscle cell: the calcium-uptake by isolated sarcotubular vesicles, and the lactic dehydrogenase with respect to amount and isozyme composition.

Materials and Methods.—*Surgical crossing of the nerves:* Crosses were made in the cat of the nerves leading to the soleus (S) and flexor digitorum (F) muscles. The techniques, and the evaluations of the purity of the crosses, were those described elsewhere.[5] The animals were the same as those mentioned.[5] A number of the experiments of that joint investigation yielded enough material to perform the additional tests to be described here. The results of the functional tests are expressed in terms of a crossover ratio, which is 1.0 in case the muscle became completely innervated by the heteronomous nerve, and 0.0 if it became self-reinnervated. We refer to Buller, Mommaerts, and Seraydarian[5] for all details.

Preparation of sarcoreticular vesicles: These were made from muscles used for the isolation of myofibrils according to Stromer *et al.*,[6] the supernatant solution being further centrifuged at 15,000 *g* for the removal of mitochondria. After centrifugation at 41,000 *g* for 90 min, we then deposited a pellet of vesicular material, which was suspended in 1 *M* sucrose. These manipulations rest upon the procedures of Seraydarian and Mommaerts,[7] but complete purification by their gradient method could not be carried through in view of the very small amounts of material in hand.

Characterization of sarcotubular vesicles: (a) EGTA-sensitivity of the ATPase (EGTA: ethylene-bis-(*N,N'*-diacetyl-β-aminoethyl)ether, a chelating agent with high specificity toward calcium ions): This followed the findings of Sreter and Gergely.[8] The medium consisted of 0.1 *M* KCl, 10-mM imidazol buffer pH 7.2, 5 mM $MgCl_2$, 5 mM ATP; 0.2

mg of vesicular protein in a total volume of 2 ml; the reaction was carried out with and without 0.5 mM EGTA. After 12 min at 25°C, the reaction was stopped with 1 ml of 5% trichloroacetic acid, and the inorganic phosphate determined in 2 ml of filtrate. (b) Calcium uptake: This follows the procedure of Hasselbach and Seraydarian.[9] The medium was 0.1 M KCl, 10-mM imidazol buffer pH 7.0, 5 mM K-oxalate, 5 mM ATP, 0.5 mM $CaCl_2$ with ^{45}Ca, 0.55 mM EGTA; 0.2 mg of vesicular protein in 2 ml; initiation of the reaction by addition of ATP. After 12 min at 25°C, the vesicles were removed by the Millipore filtration method of Martonosi and Feretos,[10] and the calcium uptake established by radioactivity measurement.

Lactic dehydrogenase: This was investigated in the supernatant solution remaining after the ultracentrifugal sedimentation of the sarcoreticular vesicles. (a) Characterization of the isozyme patterns: This was done by agar gel electrophoresis according to Yakulis et al.[11] as modified by Kar and Pearson.[12] (b) Quantitative assay: This followed the procedure of Amador et al.,[13] taking the reaction in the direction of lactate to pyruvate.

Results.—Basis of comparison: The experimental muscles were those which were cross-innervated as described. The controls were the identical contralateral muscles of which the nerves had also been sectioned, but reconnected upon themselves; these are identical to unoperated muscles in all respects tested.[5]

Differences in the properties of sarcoreticular vesicles: In accordance with the study of Sreter and Gergely[8] on "white" vs. "red" muscles, these differ in two respects. Under identical standard conditions, the vesicles from F-muscles take up calcium ions more effectively than those from S-muscles (Fig. 1); and the ATPase-activity of F-vesicles, measured without the separate addition of bulk calcium, is markedly depressed by EGTA, whereas that of S-vesicles, which can have a lesser or equal rate, is not (Table 1).

In considering these differences, it is to be kept in mind that we observe them only in the isolated preparations which are derived from the natural structures by fragmentation. They may be differently altered by the homogenization and isolation procedures. As to the meaning of the EGTA-sensitivity, this is entirely unclear. As to the difference in calcium accumulation, it would not be possible to say whether it is due to a difference in leakiness or other properties in the isolated preparation, or whether it reflects a true difference in calcium-concentrating activity in the living tissue.

FIG. 1.—Calcium uptake by sarcotubular vesicles, as modified by cross-innervation. At left for the cross F → S, uptake amounts in μmols Ca^{++} per mg sarcoreticular protein plotted as a function of the degree of transformation of the muscle. For each experiment, the point of origin of the arrow gives the measurement in the contralateral self-reinnervated control. The symbols give the measurement in the material from crossed muscle. At right, *ibid.* for the cross S → F. *Round symbols*, 6 months; *square symbols*, 12 months postoperatively.
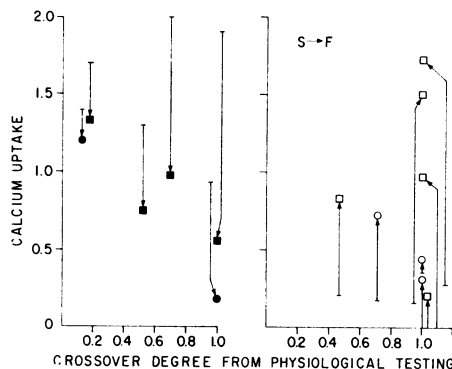
Calcium uptake is expressed in μmols per mg of vesicular protein. In exps. 3, 5 and 6, this was determined after 8 min of incubation at 27°C, in the other cases after 12 min, but in either event this would have been close to the plateau-value for F-vesicles under these circumstances.

TABLE 1. Effect of EGTA upon the ATPase of sarcotubular vesicles, μmols per mg protein per minute. Under each heading, column A gives the ATPase as such, B with EGTA, and C the residual activity expressed as the fraction of the original. One compares the respective C columns to judge the change in EGTA-sensitivity, and in the columns "Evaluation of Crossing" this is compared with the crossover percentage. The latter is derived from physiological measurements (ref. 5) which generally parallel the myosin-ATPase changes. However, in the F → S cross of cat 6, the two values diverge, and the vesicular ATPase change is well correlated with the myosin change.

Cat no.	F-muscle			F → S Crossing			Fraction	Evaluation of Crossing — EGTA effect	S-muscle			S → F Crossing			Fraction	Evaluation of Crossing — EGTA effect
	A	B	C	A	B	C			A	B	C	A	B	C		
3	0.30	0.08	0.27	0.41	0.12	0.30	0.1	Accordingly no change	0.26	0.25	0.95	0.13	0.12	0.92	0.7	Paradoxically no change
5	0.39	0.14	0.36	0.50	0.56	1.12	1.0	Completely abolished	0.35	0.39	1.12	0.15	0.16	1.04	1.0	Ibid.
6	0.61	0.11	0.18	0.45	0.18	0.40	0.9 / 0.26	Change fits better with second fig.	0.21	0.20	0.97	0.23	0.19	0.82	1.0	Ibid.
8	0.93	0.12	0.13	0.23	0.17	0.74	1.0	Strongly changed	0.20	0.19	0.96	0.33	0.17	0.52	1.0	Strongly changed
9	0.77	0.16	0.21	0.51	0.25	0.49	0.7	Accordingly changed	0.42	0.40	0.95	0.38	0.21	0.56	1.0	Ibid.
10	0.55	0.14	0.25	0.49	0.37	0.75	1.0	Strongly changed	0.30	0.29	0.94	0.34	0.20	0.60	1.0	Ibid.
12	0.55	0.13	0.23	0.57	0.17	0.29	0.5	Almost unchanged	0.20	0.20	0.98	0.32	0.26	0.80	1.0	Little change
13	0.67	0.08	0.12	0.67	0.10	0.15	0.2	Accordingly no change	0.14	0.10	0.72	0.14	0.12	0.85	0.4	No appropriate change but low accuracy

Changes in sarcoreticular vesicles found after nerve crossing: This is shown in Figure 1. Commensurate with the degree of crossing, there is a transformation of the calcium transporting activity in the direction of that of the opposite muscle type, both in the F → S and the S → F crosses, which, within the limits of accuracy, is quite pronounced, except that in most instances the S → F transition showed little change after six months. In one instance, cat 6, there was but little change in the F → S transition, but the control value was unusually low here. Concerning the EGTA sensitivity (Table 1), there tends to be the appropriate change in the F → S crosses (in cat 6, there is a better correlation with its myosin change[5] which deviated markedly from the physiologically assessed crossing); in the S → F cross, there was practically no change after six months, but a trend of incomplete changes after 12 months.

Differences in the amount and type of lactic dehydrogenase: It is found (Table 2) that the F-muscles contain vastly more lactic dehydrogenase than the S-muscles. They differ, also, in isozyme composition. The F-muscles contain predominantly the muscle or M-type, the S-muscles the heart or H-type of lactic dehydrogenase, following the distinctions made by, e.g., Markert and Møller,[14] and Wieland and Pfleiderer[15, 16] (Fig. 2).

Changes in lactic dehydrogenase found after nerve crossing: Table 2 also documents data on the quantitative assays performed in the cross-innervated muscles, and Figure 3 correlates these findings with the physiologically determined crossing ratios. It is found that in the F → S transformation, there is a far-going change in the lactic dehydrogenase contents (Fig. 3*A*), and this, furthermore, is well correlated with the ATPase transformation[5] (Fig. 3*B*). The results show, however, that the adaptation is not quite as complete as that of the ATPase, the correlation between the two being of the order of 0.75. In the S → F transformation, however, the transformation appears to be negligible after six months, and detectable though still small after 12 months postoperatively (Fig. 3*A*, *open symbols*).

Independently of these findings with respect to the amount of lactic dehydrogenase, Figure 2 shows a considerable, though incomplete, transformation of the isozyme composition in both directions.

Compare Guth, Watson, and Brown[17] for some other data on lactic dehydrogenase not directly comparable to ours.

Discussion.—Adding the results obtained here to those of Buller, Mommaerts,

TABLE 2. *Lactic dehydrogenase, quantititative assays.*

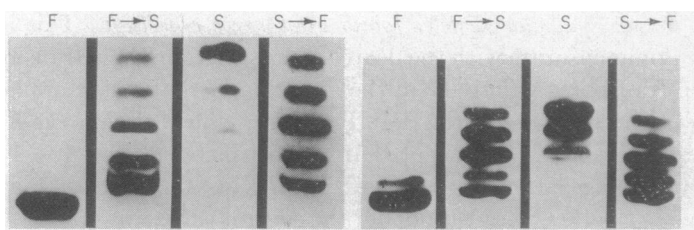|	Enzyme Units Per mg of Protein in Extract			
Cat no.	F	F → S	S	S → F
1	16,950	7,850	1,538	1,920
3	36,250	18,300	5,380	5,270
5	12,500	4,540	1,615	1,640
6	16,600	8,520	870	1,515
7	10,250	9,040	1,070	1,940
8	11,840	5,720	1,200	3,400
9	11,760	7,240	800	3,090
10	10,920	4,400	1,165	2,320
11	11,600	2,440	1,195	2,625
12	13,700	8,560	1,235	4,020

FIG. 2.—Isozyme patterns of the lactic dehydrogenase, for the muscles as identified. The left-hand set applies to a 6-months, the right-hand to a 12-months experiment; the right-hand experiment had a heavier application of sample, and a shorter running time.

and Seraydarian,[5] we have data relevant to three classes of physiological phenomena. The changes in myosin- and myofibrillar ATPase[5] relate to the neurogenically caused transformation of contraction velocity,[1-3] which they explain. The present findings have other implications.

With respect to the sarcoreticular vesicles, we are referring to a material derived from the cellular system dealing with excitation-contraction coupling (see Ebashi and Endo[18]). It would be tempting to conclude that the calcium-sequestering activity of the isolated vesicles indicates the effectiveness of the sarcoreticular system *in vivo* to concentrate these ions and so to terminate the active state. If this were so, our results would indicate a fundamental explanation of the neurogenically determined duration of the contraction cycle described by Buller and Lewis,[3] whereby we would have elucidated the second functional feature of the nerve-crossing experiments. There are two limitations which prohibit that conclusion. First, the persistence time of the excitatorily liberated calcium (cf. Jöbsis and O'Connor[19]) is likely to depend on the amount and extent of sarcoplasmic reticulum as much as on its activity per weight or surface unit. Secondly, the demonstrable transport activity may alter during the isolation procedures, e.g., S-vesicles may suffer more than F-vesicles from disintegration;
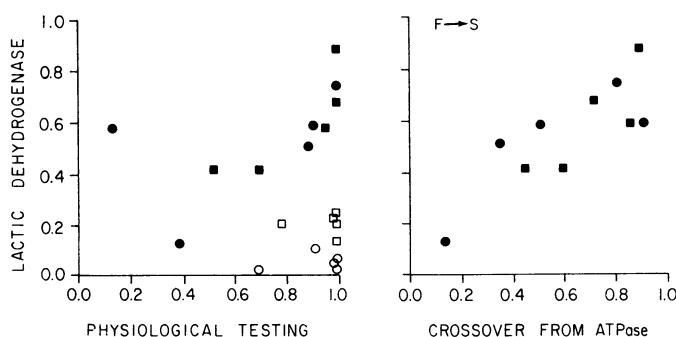

FIG. 3.—Amounts of lactic dehydrogenase as changed by nerve crossing. Fig. 3*A* (left): The abscissa shows the degree of conversion as established physiologically, the ordinate according to the change in lactic dehydrogenase amount. *Round and square symbols*, 6- and 12-months experiments, respectively. *Black symbols*, F → S; *open symbols*, S → F. Fig. 3*B* (right): Correlation, for the F → S crossing, between the degree of conversion of the lactic dehydrogenase with that of the myosin- and myofibrillar ATPase (from ref. 5).

perhaps they might heal less completely and therefore be more "leaky" during the assay. We are developing other approaches to this problem. Meanwhile, the results stand descriptively; they show that the sarcoreticular system changes upon changing the innervation in some of its properties, but the indicated far-reaching physiological conclusion is not yet allowed.

In the case of the lactic dehydrogenase, we are further removed from the contraction cycle itself. We are dealing with a metabolic provision which allows the F-muscle to carry out bursts of intense activity while engaging an oxygen debt based upon glycolysis (the M-isozyme acting in the direction from pyruvate to lactate). In the steadily active S-muscle, this provision plays no role, and the H-isozyme is a dehydrogenase oxidizing lactate, as one among possible fuels, to pyruvate. Our results show that in the F → S cross, the muscle loses the enzyme which, in its newly dictated function, it would not need. But in the opposite cross, the expected positive adaptation does not occur. Thus, the muscle would not be able to function fully in accordance with its new innervation, which might well be a handicap in competitive ecological situations. The result simply means that, while the cross-innervation experiments suggest a considerable plasticity in the development of the neural and neuromuscular interaction pattern, the flexibility allowed by the genetic potential is not unlimited. There will be great diversity as to the extent to which individual functions and their molecular bases are modifiable.

The general implications of our direction of work have been set f orth by Buller, Mommaerts, and Seraydarian.[5] We reiterate that we are showing an interference by the innervation in the chain of events from the genomic potential to the final functional differentiation.

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